

WHAT IS CLAIMED IS:

1 1. A method of forming a peptide conjugate comprising a covalent
2 linkage between a modifying group and a glycosylated or non-glycosylated peptide, wherein
3 said modifying group is conjugated to the peptide via a glycosyl linking group interposed
4 between and covalently linked to both said peptide and said modifying group, said method
5 comprising:

6 a. contacting a cell with a modified sugar comprising a sugar moiety and at
7 least one modifying group, wherein said modifying group is a member independently
8 selected from the group consisting of a water-soluble polymer, a therapeutic moiety, a
9 detectable label, a biomolecule and a targeting moiety;

10 b. incubating said cell under conditions in which said cell internalizes said
11 modified sugar;

12 c. after step b, intracellularly contacting said modified sugar with a
13 glycosylated or non-glycosylated peptide and a glycosyltransferase for which said modified
14 sugar is a substrate, thereby forming said peptide conjugate.

1 2. The method of claim 1, further comprising, after step b and before step
2 c, intracellularly contacting said modified sugar with a nucleotide and a nucleotidyl
3 transferase, thereby forming a modified nucleotide sugar, wherein
4 said modified sugar in step c is said modified nucleotide sugar.

1 3. The method of claim 1, further comprising isolating said peptide
2 conjugate.

1 4. The method of claim 1, wherein said modified sugar is a modified
2 nucleotide sugar.

1 5. The method of claim 1, wherein said modified sugar is a modified
2 activated sugar.

1 6. The method of claim 1, wherein said glycosyl linking group is an intact
2 glycosyl linking group.

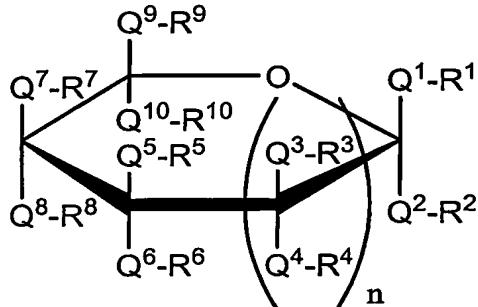
1 7. The method of claim 1, wherein said modified sugar is a precursor
 2 modified sugar that is intracellularly converted to an intermediate modified sugar by cellular
 3 enzymes after step b and before step c.

1 8. The method of claim 7, wherein said intermediate modified sugar is a
 2 phosphorylated modified sugar, wherein said phosphorylated modified sugar is formed by
 3 intracellularly contacting said modified sugar with a kinase for which said modified sugar is a
 4 substrate, thereby forming a phosphorylated modified nucleotide sugar.

1 9. The method of claim 1, wherein said water-soluble polymer comprises
 2 poly(ethylene glycol).

1 10. The method of claim 10, wherein said poly(ethylene glycol) has a
 2 molecular weight distribution that is essentially homodisperse.

1 11. The method of claim 1, wherein said modified sugar has the formula



(I)

2 wherein,

3 n represents an integer from 0 to 1;

4 Q¹, Q², Q³, Q⁴, Q⁵, Q⁶, Q⁷, Q⁸, Q⁹, and Q¹⁰ are members independently
 5 selected from a bond, substituted or unsubstituted alkylene, substituted or
 6 unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene,
 7 substituted or unsubstituted heterocycloalkylene, substituted or
 8 unsubstituted arylene, substituted or unsubstituted heteroarylene, -O-,
 9 -N(R^{1A})-, -S-, -C(O)-, and -CH₂-; wherein
 10 R^{1A} is a member selected from hydrogen, substituted or unsubstituted
 11 alkyl, substituted or unsubstituted heteroalkyl, substituted or
 12 unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl,
 13

12. The method of claim 11, wherein

$Q^1\text{-}R^1$, $Q^2\text{-}R^2$, $Q^3\text{-}R^3$, $Q^4\text{-}R^4$, $Q^5\text{-}R^5$, $Q^6\text{-}R^6$, $Q^7\text{-}R^7$, $Q^8\text{-}R^8$, $Q^9\text{-}R^9$, and $Q^{10}\text{-}R^{10}$ are members independently selected from hydrogen, $-\text{OPO}_3\text{H}_2$, $-\text{OH}$, $-\text{OCH}_3$, $-\text{CH}_3$, $-\text{C(O)H}$, $-\text{CH}_2\text{OH}$, $-\text{NHR}^{11}$, $-\text{O-CH(CH}_3\text{)}\text{COOR}^{12}$, $-\text{C(O)OR}^{13}$, $-\text{CHR}^{14}\text{-CHR}^{15}\text{-CH}_2\text{R}^{16}$, an activated leaving group, a nucleotidyl moiety and $-\text{L-M}$, wherein at least one of R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , R^9 , and R^{10} is $-\text{L-M}$, wherein

L is a linker independently selected from a bond, substituted or unsubstituted alkylene, substituted or unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkylene, substituted or unsubstituted arylene, substituted or unsubstituted heteroarylene, $-\text{O-}$, $-\text{NH-}$, $-\text{S-}$, and $\text{CH}_2\text{-}$,

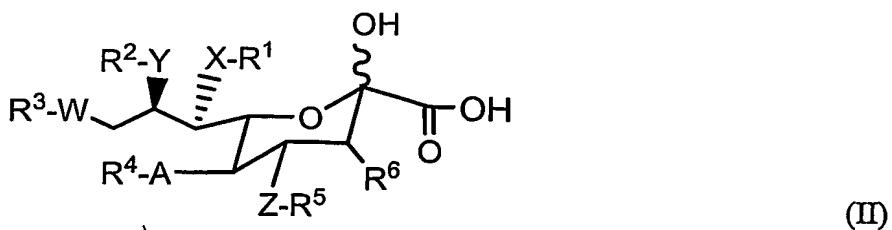
M is a modifying group, and

R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , and R^{16} are independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, and $-\text{L}^1\text{-M}^1$, wherein

L^1 is a linker independently selected from a bond, substituted or unsubstituted alkylene, substituted or unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkylene, substituted or unsubstituted arylene, substituted or unsubstituted heteroarylene, $-\text{O-}$, $-\text{NH-}$, $-\text{S-}$, and $\text{CH}_2\text{-}$, and

M^1 is modifying group.

1 13. The method of claim 11, wherein said modified sugar has the formula



2 wherein,

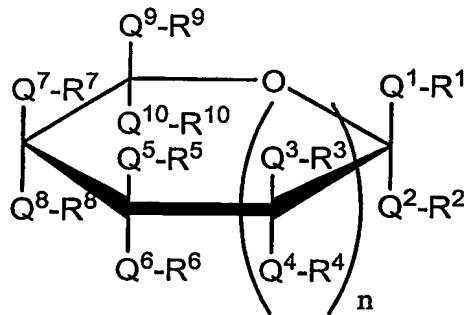
3 W, X, Y, Z, and A are members independently selected from a bond,
 4 substituted or unsubstituted alkylene, substituted or unsubstituted
 5 heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or
 6 unsubstituted heterocycloalkylene, substituted or unsubstituted arylene,
 7 substituted or unsubstituted heteroarylene, -O-, -N(R⁷)-, -S-, and -CH₂-,
 8 wherein,

9 R⁷ is a member independently selected from hydrogen, substituted or
 10 unsubstituted alkyl, substituted or unsubstituted heteroalkyl,
 11 substituted or unsubstituted cycloalkyl, substituted or unsubstituted
 12 heterocycloalkyl, substituted or unsubstituted aryl, and substituted or
 13 unsubstituted heteroaryl; and

14 R¹, R², R³, R⁴, R⁵ and R⁶ are members independently selected from -OH, -
 15 NH₂, -SH, hydrogen, substituted or unsubstituted alkyl, substituted or
 16 unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl,
 17 substituted or unsubstituted heterocycloalkyl, substituted or
 18 unsubstituted aryl, substituted or unsubstituted heteroaryl, and a
 19 modifying group, wherein at least one or R¹, R², R³, R⁴, R⁵ and R⁶ is a
 20 modifying group.

1 14. The method of claim 4, wherein said modified nucleotide sugar has the

2 formula



4 wherein,

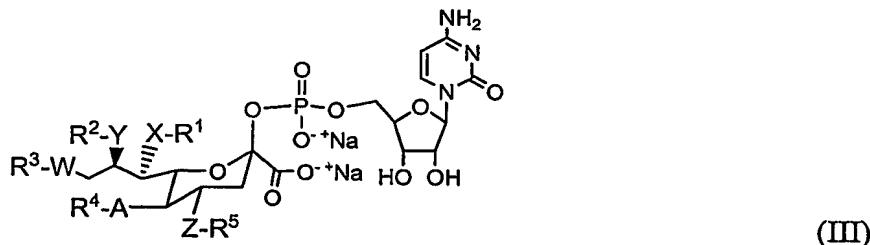
5 n represents an integer from 0 to 1;

6 Q¹, Q², Q³, Q⁴, Q⁵, Q⁶, Q⁷, Q⁸, Q⁹, and Q¹⁰ are members independently selected from a bond, substituted or unsubstituted alkylene, substituted or unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkylene, substituted or unsubstituted arylene, substituted or unsubstituted heteroarylene, -O-, -N(R^{1A})-, -S-, -C(O)-, and -CH₂-; wherein

7 R^{1A} is a member selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl; and

8 R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸, R⁹, and R¹⁰ are members independently selected from -OPO₃H₂, -OH, -NH₂, -SH, hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, an activated leaving group, a nucleotidyl moiety, and a modifying group, wherein at least one of R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸, R⁹, and R¹⁰ is a modifying group and a nucleotidyl moiety.

1 15. The method of claim 14, wherein said modified nucleotide sugar has
2 the formula



3 wherein,

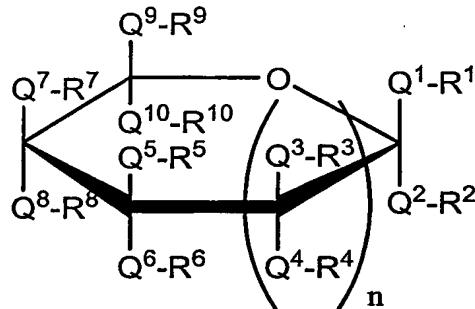
4 W, X, Y, Z, and A are members independently selected from a bond,
5 substituted or unsubstituted alkylene, substituted or unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkylene, substituted or unsubstituted arylene,

9 substituted or unsubstituted heteroarylene, -O-, -N(R⁷)-, -S-, and -CH₂-,
 10 wherein,

11 R⁷ is a member independently selected from hydrogen, substituted or
 12 unsubstituted alkyl, substituted or unsubstituted heteroalkyl,
 13 substituted or unsubstituted cycloalkyl, substituted or unsubstituted
 14 heterocycloalkyl, substituted or unsubstituted aryl, and substituted or
 15 unsubstituted heteroaryl; and

16 R¹, R², R³, R⁴, and R⁵ are independently selected from -OH, -NH₂, -SH,
 17 hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted
 18 heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or
 19 unsubstituted heterocycloalkyl, substituted or unsubstituted aryl,
 20 substituted or unsubstituted heteroaryl, and a modifying group, wherein at
 21 least one or R¹, R², R³, R⁴, and R⁵ is a modifying group.

1 16. The method of claim 5, wherein said modified nucleotide sugar has the
 2 formula



(I)

3 wherein,

4 n represents an integer from 0 to 1;

5 Q¹, Q², Q³, Q⁴, Q⁵, Q⁶, Q⁷, Q⁸, Q⁹, and Q¹⁰ are members independently
 6 selected from a bond, substituted or unsubstituted alkylene, substituted or
 7 unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene,
 8 substituted or unsubstituted heterocycloalkylene, substituted or
 9 unsubstituted arylene, substituted or unsubstituted heteroarylene, -O-,
 10 -N(R^{1A})-, -S-, -C(O)-, and -CH₂-, wherein

11 R^{1A} is a member selected from hydrogen, substituted or unsubstituted
 12 alkyl, substituted or unsubstituted heteroalkyl, substituted or
 13 unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl,
 14

15 substituted or unsubstituted aryl, and substituted or unsubstituted
16 heteroaryl; and
17 R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸, R⁹, and R¹⁰ are members independently
18 selected from -OPO₃H₂, -OH, -NH₂, -SH, hydrogen, substituted or
19 unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted
20 or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl,
21 substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl,
22 an activated leaving group, a nucleotidyl moiety, and a modifying group,
23 wherein at least one of R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸, R⁹, and R¹⁰ is a
24 modifying group and an activated leaving group.

1 17. The method of claim 1, wherein said peptide is selected from the group
2 consisting of granulocyte colony stimulating factor, interferon-alpha, interferon-beta, Factor
3 VIIa, Factor IX, follicle stimulating hormone, erythropoietin, granulocyte macrophage colony
4 stimulating factor, interferon-gamma, alpha-1-protease inhibitor, glucocerebrosidase, tissue
5 plasminogen activator protein, interleukin-2, Factor VIII, chimeric tumor necrosis factor
6 receptor, urokinase, chimeric anti-glycoprotein IIb/IIIa antibody, chimeric anti-HER2
7 antibody, chimeric anti-respiratory syncytial virus antibody, chimeric anti-CD20 antibody,
8 DNase, chimeric anti-tumor necrosis factor antibody, human insulin, hepatitis B sAg,
9 interferon-omega, alpha-galactosidase A, alpha-iduronidase, anti-thrombin III, human
10 chorionic gonadotropin, and human growth hormone.

1 18. A cell comprising a peptide conjugate, said peptide conjugate
2 comprising:
3 (i) a modifying group and a peptide, wherein said modifying group is linked to said
4 peptide via a glycosyl linking group interposed between and covalently linked
5 to both the peptide and said modifying group; and
6 (ii) said modifying group is a member independently selected from the group
7 consisting of a water-soluble polymer, a therapeutic moiety, a detectable label,
8 and a targeting moiety.
1 19. The method of claim 18, wherein said glycosyl linking group is an
2 intact glycosyl linking group.